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Development of Environmental Health Criteria for Insensitive Munitions: Aquatic Ecotoxicological Exposures Using 2,4-Dinitroanisole

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Jennifer G. Laird, Bob Winstead, Sandra M. Brasfield,
and Mark S. Johnson

March 2013

Environmental Laboratory

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Final report

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Abstract

Insensitive munition formulation (IMX)-101 consists of 2,4-dinitroanisole (DNAN), 3-nitro-1,2,4-triazol-5-one (NTO), and nitroguanidine (NQ). While general aquatic ecotoxicological information is available for two of the IMX constituents (NTO and NQ), such data are not known to be available for DNAN. Thus, acute and chronic aquatic toxicity bioassays were conducted using standard fish (*Pimephales promelas*) and invertebrate (*Ceriodaphnia dubia*) models. Chemical analysis of test water indicated that DNAN concentrations were relatively stable during the bioassays. Acute toxicity was similar for the two species tested, with 48-hr lethal median concentrations (LC50) ranging from 37 to 42 mg/L DNAN. Chronic toxicity tests indicated that fish survival (7-day LC50 = 10 mg/L) was significantly more sensitive to DNAN relative to the invertebrate (no significant impact on survival at 24 mg/L). However, the reproduction endpoint for the invertebrate was significantly more sensitive to DNAN than survival. When assessing the most sensitive chronic endpoints, the two test species indicated similar chronic toxicity, with lowest observable adverse impacts ranging from 10 to 12 mg/L DNAN and median effects on sublethal endpoints (growth, reproduction) ranging from 11 to 15 mg/L DNAN. Chronic no-effect concentrations ranged from approximately 6 to 8 mg/L DNAN.

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Preface

This study was conducted for the Strategic Environmental Research and Development Program (SERDP) under Project Number ER 2223, "Development of Environmental Health Criteria for Insensitive Munitions (IMX-101-104)." The principal investigator was Dr. Mark Johnson, U.S. Army Institute of Public Health (AIPH, MCHB-IP-THE Toxicology), Aberdeen Proving Ground, MD.

The work was performed by the Environmental Risk Assessment Branch (EP-R) of the Environmental Processes and Engineering Division (EP), U.S. Army Engineer Research and Development Center – Environmental Laboratory (ERDC-EL). At the time of publication, Buddy Goatcher was Chief, CEERD-EP-R; Warren Lorentz was Chief, CEERD-EP; and Dr. Elizabeth Ferguson was the Technical Director for Military Environmental Engineering and Science. The Deputy Director of ERDC-EL was Dr. Jack Davis and the Director was Dr. Beth Fleming.

COL Kevin J. Wilson was the Commander of ERDC, and Dr. Jeffery P. Holland was the Director.

1 Introduction

An effective environmental management strategy for wastewater discharges is dependent on accurate risk assessment to minimize potential consequences of anthropogenic impacts on ecosystem health. The environmental impacts of munitions and explosives of concern are difficult to predict due to the limited information available and rapid degradation to toxic products. Insensitive munition (IMX)-101 was recently approved as the main fill ingredient in M795 155-mm artillery munitions with over 20,000 lb[1] manufactured (Fung et al. 2009). This relatively new IM mixture consists of 2,4-dinitroanisole (DNAN), 3-nitro-1,2,4-triazol-5-one (NTO), and nitroguanidine (NQ).

Given its current production, use and stability (Bausinger and Preuss 2009), DNAN has the potential to be classified as an environmental contaminant upon release during manufacturing, testing, training, and use. While some aquatic ecotoxicology information exists for the other IMX-101 constituents NTO (S&ME 2007; Haley et al. 2009; Sayers 2009a,b) and NQ (van der Schalie 1985), there is currently no known information on the aquatic ecotoxicology of DNAN. Consequently, the aquatic ecotoxicology of DNAN is the focus of this report.

This document provides a brief status report on the acute and chronic bioassays conducted at the U.S. Army Engineer Research and Development Center (ERDC) to generate aquatic toxicity reference values specific to DNAN.

[1] Equivalent to 9,072 kg.

2 Methods

DNAN preparation

DNAN was acquired from Holston Army Ammunition Plant (Bob Winstead, BAE Systems, Kingsport, Tennessee, USA). DNAN solutions were prepared by 48-hr magnetic stirring in the dark. Alternative methods were evaluated involving solvent carriers (acetonitrile, methanol)[1] but were not employed since these carriers induced unacceptable sublethal toxicity to *Ceriodaphnia dubia*. Solubilized DNAN in less toxic solvent carriers (acetone, ethanol) led to re-crystallization upon spiking into moderately hard reconstituted water (MHRW). Dissolution of DNAN by bath sonication into MHRW was successful but was not used due to the resulting yellow coloration of the test medium. The yellow coloration may suggest presence of DNAN degradation compounds, such as dinitrophenol. Breakdown compounds with -OH and $-NO_2$ functional groups (e.g., dinitrophenol) were previously predicted in the alkaline hydrolysis of DNAN (Hill et al. 2012, Koutsospyros et al. 2012).

Acute toxicity testing

Acute (48-hr) toxicity tests employed the larval fish *Pimephales promelas* and the water flea *Ceriodaphnia dubia*. All *P. promelas* were obtained from Aquatic Biosystems (Fort Collins, Colorado, USA) while *C. dubia* were obtained from ERDC in-house cultures (originally ECTesting, Superior, Wisconsin, USA). Tests were conducted in accordance with standard guidance (U.S. Environmental Protection Agency (USEPA) 2002a). Initially, 48-hr DNAN range-finding toxicity tests (concentrations: 5, replicates: 3) were conducted for both *P. promelas* and *C. dubia*. MHRW, used as the diluent and control water, was formulated according to USEPA (2002a) to hardness and alkalinity levels of 80 and 60 mg/L as $CaCO_3$, respectively. Organisms were initially exposed to a nominal concentration range of 0-100 mg/L DNAN in MHRW, using a 90% dilution series (100, 10, 1, 0.1, 0.01%). Based on range-finding results, definitive acute toxicity tests were conducted (concentrations: 5, replicates: 4) for both test species in the nominal concentration range of 0–100 mg/L DNAN, using a 50% dilution series (100, 50, 25, 13, 6%). The measurement endpoint was survival after 48-hr exposure. Analytical samples from the acute definitive bioassays were

[1] Solvent carriers were spiked into water without employing methodology to evaporate the carrier.

submitted to the U.S. Army Institute of Public Health (AIPH), Laboratory Sciences Portfolio (POC: Mr. David F. Morrow).

Chronic toxicity testing

Chronic (7-day) toxicity tests employed *P. promelas* and *C. dubia* in accordance with USEPA (2002b). Nominal concentrations ranged from 0–25 mg/L, selected based on the results of the definitive acute tests, using a 50% dilution series (100, 50, 25, 13, 6, 3%). Both chronic test methods involved daily, static water renewals (80% renewal for *P. promelas*, 100% renewal for *C. dubia*) using a fresh DNAN solution, prepared as described previously. The *P. promelas* test method used < 24-hr-old larval fish and a twice-daily feeding ration of *Artemia sp.* naulpii. The fish test involved six concentrations and a control (MHRW), consisting of five replicates each (concentrations: 6, replicates: 5). Survival, biomass, and growth (dry weight basis) were assessed following 7 days of exposure. The *C. dubia* bioassay is a three-brood test; thus, the duration can potentially range from 6 to 8 days. According to guidance, *C. dubia* bioassays can be terminated once 60% of the controls have three broods of neonates. However, it was ensured that 100% of the controls had a third brood prior to termination. The *C. dubia* test involved six concentrations and a control, consisting of ten replicates each (concentrations: 6, replicates: 10). A daily feeding ration of 1:1 *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) and YCT (yeast, cereal leaves, and trout chow fish food) was supplied. Measurement endpoints for the *C. dubia* test were survival and reproduction. For both chronic tests, in-water and out-water samples were collected and submitted in three batches to (AIPH) to ensure that the 7-day sample holding time was not exceeded.

Reference toxicity testing

The selected reference toxicant for *P. promelas* and *C. dubia* was potassium chloride (KCl). Reagent grade KCl was weighed and completely dissolved in MHRW. Five triplicated concentrations were prepared (100, 50, 25, 12.5, 6.25%) with the number of organisms in each replicate previously described. The 100% concentration was 2.7 g/L for *Pimephales promelas* and 1.0 g/L for *Ceriodaphnia dubia*. Test endpoints measured were the same as previously described in the acute and chronic testing sections.

Statistical analysis

Survival data were arcsine square-root transformed prior to statistical analysis. Data normality (Kolmogorov–Smirnov test), homogeneity (Levene's test), and treatment differences were compared to the reference (one way ANOVA and Dunn's or Dunnett's Methods) and statistical significance was determined at $\alpha = 0.05$ using SigmaStat software (SPSS, Chicago, Illinois, USA). When normality was not achieved, the non-parametric Kruskal–Wallis one-way ANOVA on ranks was applied. The lethal median concentration producing 50% mortality (LC50) and inhibition concentrations (IC50, IC25) for sublethal endpoints were determined by the trimmed Spearman–Karber method (Toxcalc®, Version 5.0, Tidepool Scientific Software, McKinleyville, California). The LC50 and IC25 values are presented with 95% confidence limits in parentheses. No observable effect concentrations (NOEC) and lowest observable effect concentrations (LOEC) were also calculated using ToxCalc v5.0 and confirmed by one-way ANOVA and Dunn's or Dunnett's Methods. Maximum allowable concentrations (MATC), also known as the chronic value (ChV), were calculated as the geometric mean of the NOEC and LOEC. The acute-to-chronic ratio (ACR) was calculated as the 48-hr LC50 divided by the MATC.

Analytical chemistry

Water samples (40 mL) were collected by ERDC-EL and sent to the Army Institute of Public Health (AIPH) for analysis. The samples were maintained at 4 °C in dark conditions at all times. A 10-mL water aliquot was extracted from each sample using 2 mL of isoamyl acetate while shaking on a flatbed shaker for 1 hr. After separation, the isoamy acetate layer was placed in an auto-sampler vial and stored at 4 °C until analysis. All samples were extracted the day after receipt, with one exception; six samples collected on 1 February 2012 associated with the acute toxicity tests were one day beyond the 7-day holding time. This is not expected to have any impact on the results. DNAN (Lot # BAE10H281-008) used for calibration and quantification was obtained from BAE Systems (Ordnance Systems, 4509 West Stone Drive, Kingsport, TN 37660). DNAN used for quality control samples (LCS-LCS Duplicate) was obtained from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, 63103). The standards used to calibrate the analytical analysis system were extracted with the same water volume (10 mL) as the samples to compensate for potential differences in extraction efficiency. The samples were diluted as necessary to bring the sample concentrations within the range of the instrument calibration. Analysis was

performed using an Agilent 6890 gas chromatography (GC) fitted with an electron capture detector (ECD; Santa Clara, CA). A J&W DB-17 column was used for the primary analytical column and DB-1 was used for confirmation. The samples were reported in units of ug/mL. Laboratory control samples (LCS) and duplicates (LCSD) were analyzed with test samples and percent recoveries were within the current acceptable limit.

3 Results

Acute toxicity testing

Acceptability criteria for control survival (≥ 90%) and water quality (Appendix A; Tables A1-A4) were met for all bioassays. Acute reference toxicity tests (KCl) for *P. promelas* and *C. dubia* resulted in 48-hr LC50 values of 0.78 (95% confidence limits: 0.69 – 0.87) and 0.68 (0.62–0.74) g KCl/L, respectively. This indicates comparable sensitivity to the historic ranges in control charts (± 2 S.D. from the mean) for *P. promelas* (0.56 – 1.01 g KCl/L) and *C. dubia* (0.20–0.76 g KCl/L). Range finding tests at 10 mg/L DNAN (nominal) resulted in similar survival for both test species relative to the control. However, complete mortality was observed at 100 mg/L (nominal) DNAN (Tables 1 and 2). In the definitive acute (48-hr) toxicity testing, the concentration of DNAN during the exposure period remained relatively stable (Table 2), and all DNAN acute toxicity endpoints were based on measured concentrations (Table 3). Survival response curves for *P. promelas* and *C. dubia* were within the same dose range (Figure 1). The 48-hr LC50 trended lower for *P. promelas* (37 [33 – 41]) relative to *C. dubia* (42 [37 – 47]), suggesting slightly greater acute sensitivity to DNAN. Further, mortality in the fish was observed relatively sooner (within 2 hr of exposure) compared to *C. dubia* (within 24 to 48 hr). After 48 hr, however, the 95% confidence intervals for the two test species overlapped, indicating statistically similar sensitivity to DNAN.

Table 1. Nominal 2,4 dinitroanisole (DNAN) concentrations and survival for the acute (48-hr) range-finding *Pimephales promelas* and *Ceriodaphnia dubia* bioassays. Concentrations were not measured, as range-finding bioassays served only to determine appropriate exposure concentrations for definitive acute testing. Asterisks denote statistically significant reductions relative to the control.

Nominal (mg/L)	48-hr *Pimephales promelas* survival	48-hr *Ceriodaphnia dubia* survival
Control	100 ± 0	100 ± 0
0.01	100 ± 0	87 ± 23
0.1	100 ± 0	93 ± 12
1	100 ± 0	100 ± 0
10	100 ± 0	100 ± 0
100	0 ± 0*	0 ± 0*

Table 2. Nominal and measured 2,4 dinitroanisole (DNAN) concentrations and survival for the definitive acute (48-hr) *Pimephales promelas* (a) and *Ceriodaphnia dubia* (b) bioassays. Asterisks denote statistically significant reductions relative to the control.

Nominal (mg/L)	Measured Test Initiation (mg/L)	Measured Test Termination (mg/L)	Mean Survival (± 1 S.D.)
(a)			
0	<0.01	<0.01	100 ± 0
6	7.1	6.6	100 ± 0
12	13	14	100 ± 0
25	28	28	75 ± 6*
50	74	74	0 ± 0*
100	120	120	0 ± 0*
(b)			
0	<0.01	<0.01	90 ± 20
6	7.1	7.2	95 ± 10
12	13	14	80 ± 0
25	28	28	95 ± 10
50	74	68	0 ± 0*
100	120	120	0 ± 0*

Chronic toxicity testing

Acceptability criteria for control survival (≥ 80%; Table 4) and water quality (Appendix A; Tables A-5 and A-6) were met for bioassays testing both species. The sublethal endpoints for *P. promelas* (> 0.25 mg dry mass) and *C. dubia* (three broods of ≥ 15 neonates) also met control acceptability criteria. Chronic reference toxicity testing results were consistent for both species during the testing period (Appendix B; Table B2). The concentration of DNAN during the chronic exposures remained relatively stable (Table 5), and all DNAN chronic toxicity endpoints were based on measured concentrations averaged over the duration of the testing (Table 3). The *C. dubia* test was terminated after 6 days, since all individuals in the control achieved third brood (Table 4). As expected, the chronic bioassays provided more sensitive endpoints relative to the acute tests (Tables 3 and 4). The chronic *C. dubia* survival (LC50 > 24.2 mg/L) endpoint was substantially less sensitive than the *P. promelas* survival (LC50 = 10.0 [8.8 – 11.2] mg/L) endpoint (Table 3). However, the sublethal endpoints for both organisms were more similar in sensitivity, with chronic DNAN toxicity falling in the range of 8–15 mg/L (Table 3).

Table 3. Toxicity reference values for *Pimephales promelas* and *Ceriodaphnia dubia* exposed to 2,4-dinitroanisole. The no observable effect concentration (NOEC), lowest observable effect concentration (LOEC), maximum allowable concentration (MATC)[1], median lethal concentration (LC50), and acute-to-chronic ratio (ACR) are provided. Ninety-five percent confidence intervals for LC50 values are indicated in parentheses. The combined measure provides the most sensitive value among the tested endpoints (e.g., survival vs. growth/reproduction).

Species	Exposure Duration	Measure	Endpoint	DNAN (mg/L)
Pimephales promelas	24-h	Survival	NOEC	13
		Survival	LOEC	28
		Survival	LC50	41 (37 – 45)
	48-h	Survival	NOEC	13
		Survival	LOEC	28
		Survival	LC50	37 (33 – 41)
	7-d	Survival	NOEC	5.8
		Survival	LOEC	11.6
		Survival	LC50	10.0 (8.8 – 11.2)
		Growth	NOEC	11.6
		Growth	LOEC	24.6
		Combined	MATC / ChV	8.2
		Combined	IC25	10.4 (8.2 – 14.3)
		Combined	IC50	15.1 (12.3 – 17.7)
		Combined	ACR = 4.5	
Ceriodaphnia dubia	24	Survival	NOEC	74
		Survival	LOEC	120
		Survival	LC50	82 (72 – 93)
	48	Survival	NOEC	28
		Survival	LOEC	74
		Survival	LC50	42 (37 – 47)
	6-d	Survival	NOEC	24.2
		Survival	LOEC	>24.2
		Survival	LC50	>24.2
		Reproduction	NOEC	6.2
		Reproduction	LOEC	12.2
		Combined	MATC / ChV	8.7
		Combined	IC25	8.2 (7.4 – 8.7)
		Combined	IC50	10.6 (10.0 – 11.2)
		Combined	ACR = 4.8	

[1] Also known as chronic value (ChV).

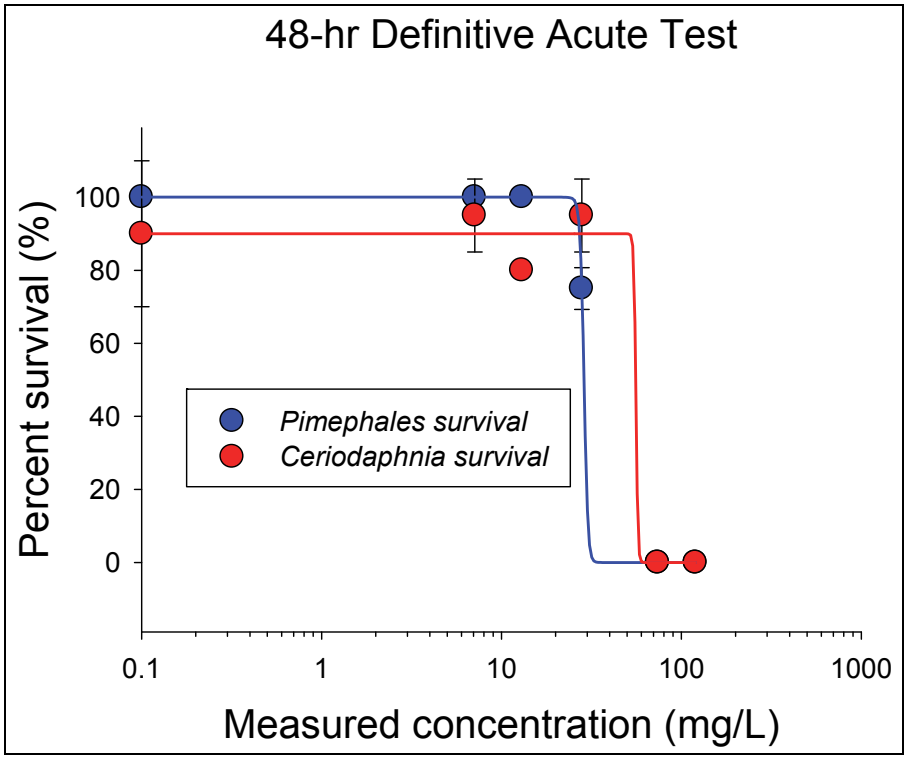

Figure 1. 48-hr dose response curves for *Pimephales promelas* and *Ceriodaphnia dubia* exposed to DNAN in definitive acute bioassay testing. Note that concentration data are plotted on a log10 scale.

Table 4. Results from the 7-day *Pimephales promelas* chronic toxicity test (a), and the three-brood *Ceriodaphnia dubia* chronic toxicity test (b). NA = Not available due to complete mortality.

(a)

Mean Measured Concentration mg/L (± 1 S.D.)	Mean Survival (± 1 S.D.)	Mean Biomass (mg) (± 1 S.D.)	Mean Growth (mg) (± 1 S.D.)
Control (0.2 ± 0.7)	98 ± 4%	0.421 ± 0.044	0.291 ± 0.044
0.7 ± 0.2	100 ± 0%	0.418 ± 0.034	0.288 ± 0.034
1.4 ± 0.5	96 ± 5%	0.454 ± 0.059	0.324 ± 0.059
2.5 ± 1.0	98 ± 4%	0.436 ± 0.074	0.306 ± 0.074
5.8 ± 1.7	90 ± 12%	0.452 ± 0.064	0.322 ± 0.064
11.6 ± 3.6	38 ± 4%*	0.300 ± 0.077*	0.170 ± 0.077*
24.6 ± 1.8	0 ± 0%*	NA	NA

(b)

Mean Measured Concentration mg/L (± 1 S.D.)	Mean Survival (± 1 S.D.)	Mean Total Reproduction (± 1 S.D.)	Mean Total Neonates/survivor (± 1 S.D.)
Control (0.0 ± 0.0)	90 ± 32%	34.8 ± 13.0	38.7 ± 4.7
0.7 ± 0.1	100 ± 0%	38.4 ± 4.6	38.4 ± 4.6
1.5 ± 0.3	100 ± 0%	38.3 ± 3.9	38.3 ± 3.9
3.1 ± 1.8	100 ± 0%	39.7 ± 5.5	39.7 ± 5.5
6.2 ± 0.5	100 ± 0%	36.2 ± 2.9	36.2 ± 2.9
12.2 ± 1.2	100 ± 0%	12.8 ± 4.6*	12.8 ± 4.6*
24.2 ± 2.0	90 ± 32%	0.2 ± 0.4*	0.1 ± 0.3*

Table 5. Nominal and measured 2,4 dinitroanisole (DNAN) concentrations provided as means (± one standard deviation from the mean) for the chronic *Pimephales promelas* (a), and *Ceriodaphnia dubia* (b), bioassays. In-water is defined as the freshly prepared DNAN water used in water exchanges while out-water is defined as the 24-hr-old water sampled prior to water renewal.

Nominal (mg/L)	Measured In-water Mean (mg/L)	Measured Out-water Mean (mg/L)	Overall Mean (in- and out-water) (mg/L)
(a)			
0	0.2 ± 0.7	0.4 ± 1.1	0.2 ± 0.7
0.8	0.7 ± 0.2	0.7 ± 0.1	0.7 ± 0.2
1.6	1.3 ± 0.6	1.4 ± 0.2	1.4 ± 0.5
3.1	2.3 ± 1.4	2.8 ± 0.2	2.5 ± 1.0
6.3	5.6 ± 2.0	5.9 ± 0.6	5.8 ± 1.7
12.5	11.0 ± 3.6	12.2 ± 2.4	11.6 ± 3.6
25.0	24.0 ± 1.9	25.3 ± 1.7	24.6 ± 1.8
(b)			
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
0.8	0.7 ± 0.2	0.7 ± 0.1	0.7 ± 0.1
1.6	1.5 ± 0.4	1.5 ± 0.2	1.5 ± 0.3
3.1	2.6 ± 1.3	3.6 ± 2.1	3.1 ± 1.8
6.3	6.4 ± 0.4	6.1 ± 0.6	6.2 ± 0.5
12.5	12.3 ± 0.8	12.0 ± 1.5	12.2 ± 1.2
25.0	24.0 ± 1.9	24.4 ± 2.3	24.2 ± 2.0

4 Discussion

Acute toxicity testing

The 48-hr acute toxicity of DNAN ranged from 37 to 42 mg/L for the two species tested (Table 3). These results place DNAN into the toxicity category of slightly toxic (Figure 2a). The cited toxicity categories were distributed by the U.S. Fish and Wildlife Service (USFWS 1984) to serve as general guidance to compare the toxicity of various chemicals and are non-regulatory. In comparison, data acquired from Talmage et al. (1999) and the USEPA's ECOTOX database (http://cfpub.epa.gov/ecotox/; queried May 2012) for other traditional munitions fall into the more toxic categories of highly toxic and moderately toxic. Also, 2,4-dinitrophenol and royal demolition explosive (RDX) are classified as slightly toxic; the acute toxicity of DNAN was less than literature-reported toxicity ranges for 2,4-dinitrophenol, RDX, dinitrobenzene, trinitrotoluene (TNT), and lead (Figure 2a).

Chronic toxicity testing

The chronic toxicity of DNAN ranged from 8.2 to 10.0 mg/L, using the most sensitive effect endpoint obtained for each of the two species tested (Table 3). The median value of all chronic toxicity effect endpoints (Table 3) places DNAN into the toxicity category of moderately toxic (USFWS 1984; Figure 2b), although most of the DNAN data distribution is in the slightly toxic category. In comparison, data acquired from Talmage et al. (1999) and the USEPA's ECOTOX database (http://cfpub.epa.gov/ecotox/; queried May 2012) for other traditional munitions fall into the more toxic categories of super toxic and highly toxic, while 2,4-dinitrophenol and RDX are also classified as moderately toxic. The chronic toxicity of DNAN was less than the literature-reported toxicity ranges for 2,4-dinitrophenol, RDX, dinitrobenzene, trinitrotoluene (TNT), and lead (Figure 2b).

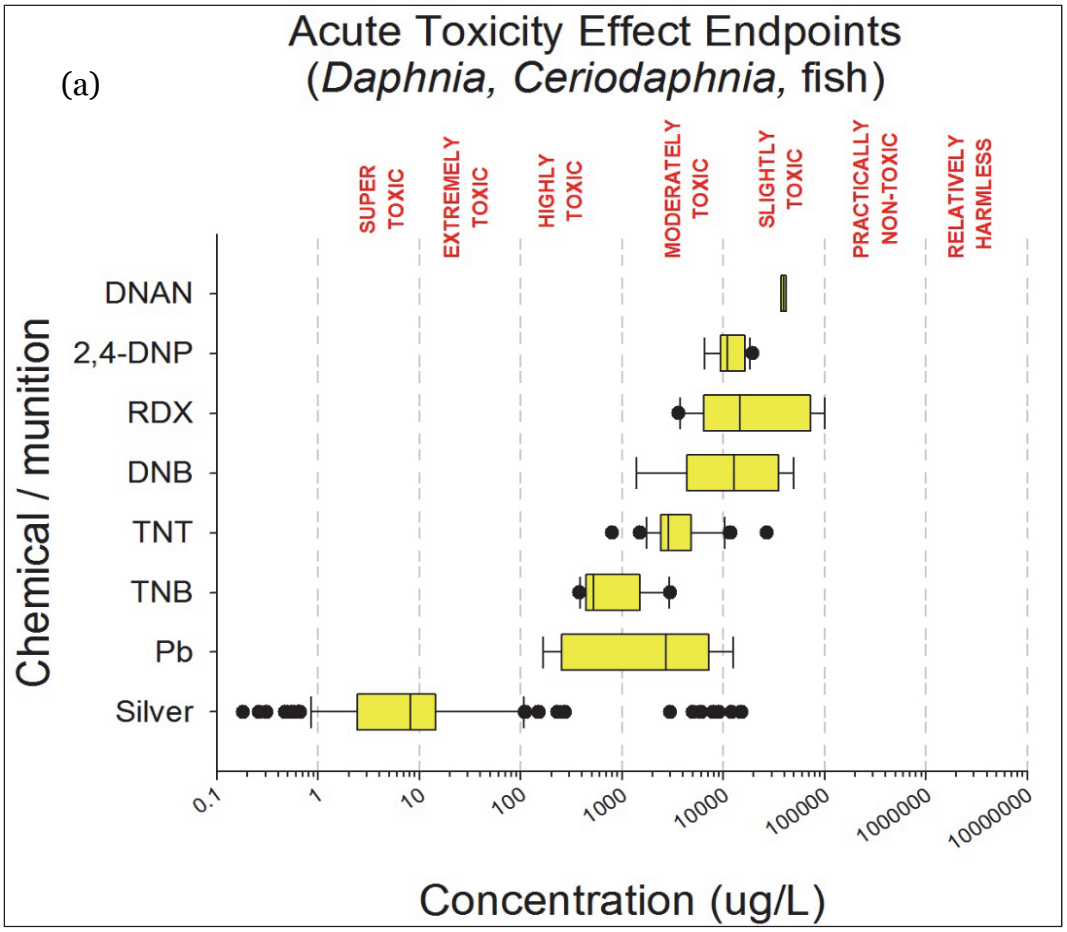

Figure 2. Comparison of toxicity reference values for 2-4 dinitroanisole (DNAN) generated in the current report to toxicity reference values for other traditional munitions (silver was included as a reference toxicant). Acute toxicity is provided in panel a, while chronic toxicity is provided in panel b. All summarized toxicity reference values are effect endpoints (e.g., LC50, IC50, LOEC, etc.) for fish, *Daphnia* and *Ceriodaphnia* species obtained from Talmage et al. (1999) and the USEPA ECOTOX database (http://cfpub.epa.gov/ecotox/). Box margins represent the 25th and 75th percentiles of the data distribution, error bars represent 10th and 90th percentiles of the data distribution (single points represent outlier data in the top and bottom 10% of the data distribution), and lines within the boxes represent the median toxicity reference value. General toxicity severity ranges (USFWS 1984) are indicated in a red font across the top of the figure. Note that the x-axis is plotted on a log10 scale. 2,4-DNP = 2,4 dinitrophenol, RDX = royal demolition explosive, DNB = dinitrobenzene, TNT = trinitrotoluene, TNB = trinitrobenzene, Pb = lead. (continued)

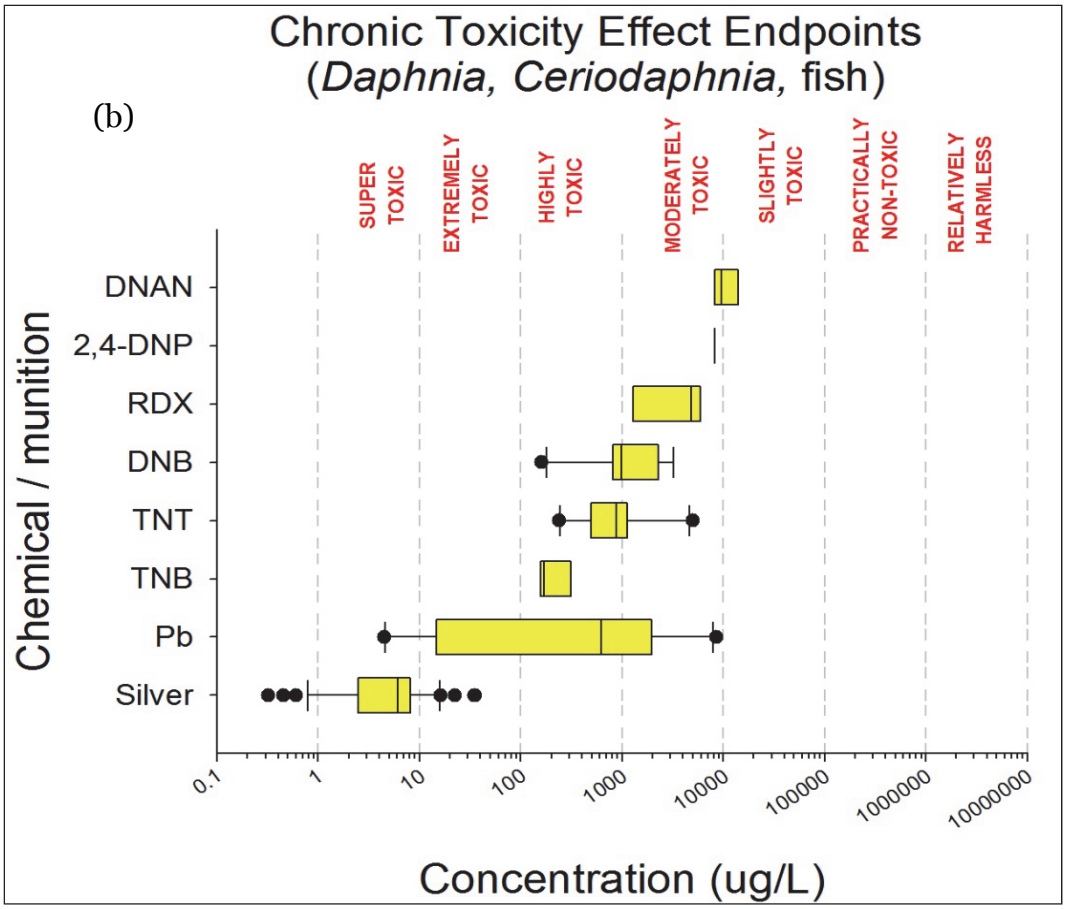

Figure 2. (concluded).

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Appendix A: Water quality parameters

Table A1. Water quality parameters for the 48-hr *Pimephales promelas* range-finder bioassay exposed to 2,4-dinitroanisole (DNAN). Means and one standard deviation from the mean are indicated, with the minimum and maximum range of the data provided in parentheses.

Nominal DNAN Treatment (%)	Nominal DNAN Concentration (mg/L)	Temperature (° C)	pH (SU)	Dissolved Oxygen (mg/L)	Conductivity (µS/cm)
Control	0	24.0 ± 0.6 (23.1 - 24.5)	7.05 ± 0.35 (6.85 - 7.58)	7.8 ± 0.2 (7.6 - 8)	284 ± 1 (283 - 285)
0.01%	0.01	24.1 ± 0.5 (23.3 - 24.4)	7.15 ± 0.42 (6.86 - 7.77)	7.7 ± 0.1 (7.6 - 7.8)	284 ± 1 (283 - 286)
0.1%	0.1	23.9 ± 0.4 (23.2 - 24.1)	7.27 ± 0.39 (7.05 - 7.85)	7.6 ± 0 (7.6 - 7.7)	286 ± 3 (283 - 290)
1%	1	23.8 ± 0.5 (23.1 - 24.1)	7.35 ± 0.37 (7.15 - 7.9)	7.7 ± 0.1 (7.6 - 7.8)	285 ± 2 (282 - 286)
10%	10	23.6 ± 0.6 (22.8 - 23.9)	7.38 ± 0.38 (7.17 - 7.95)	7.7 ± 0 (7.7 - 7.8)	286 ± 1 (285 - 287)
100%	100	23.6 ± 0.6 (22.6 - 23.9)	7.43 ± 0.38 (7.2 - 7.99)	7.8 ± 0 (7.7 - 7.8)	301 ± 2 (298 - 303)

Table A2. Water quality parameters for the 48-hr *Ceriodaphnia dubia* range-finder bioassay. Means and one standard deviation from the mean are indicated, with the minimum and maximum range of the data provided in parentheses.

Nominal Treatment (%)	Nominal Concentration (mg/L)	Temperature (° C)	pH (SU)	Dissolved Oxygen (mg/L)	Conductivity (µS/cm)
Control	0	23.5 ± 0.6 (22.9 - 24.1)	7.1 ± 0.33 (6.86 - 7.58)	8.1 ± 0.4 (7.6 - 8.3)	299 ± 21 (283 - 330)
0.01%	0.01	24 ± 0.4 (23.3 - 24.2)	7.4 ± 0.25 (7.26 - 7.77)	8.1 ± 0.3 (7.6 - 8.3)	291 ± 5 (286 - 298)
0.1%	0.1	23.8 ± 0.4 (23.2 - 24.1)	7.43 ± 0.28 (7.27 - 7.85)	8.1 ± 0.3 (7.7 - 8.2)	292 ± 4 (286 - 295)
1%	1	23.7 ± 0.4 (23.1 - 23.9)	7.49 ± 0.28 (7.32 - 7.9)	8.1 ± 0.2 (7.8 - 8.2)	297 ± 8 (286 - 303)
10%	10	23.6 ± 0.5 (22.8 - 23.9)	7.56 ± 0.26 (7.42 - 7.95)	8.1 ± 0.2 (7.8 - 8.2)	292 ± 3 (287 - 294)
100%	100	23.5 ± 0.6 (22.6 - 23.8)	7.6 ± 0.26 (7.46 - 7.99)	8.1 ± 0.2 (7.7 - 8.2)	307 ± 5 (300 - 311)

Table A3. Water quality parameters for the definitive, acute (48-hr) *Pimephales promelas* bioassay. Means and one standard deviation from the mean are indicated, with the minimum and maximum range of the data provided in parentheses.

Nominal Treatment (%)	Nominal Concentration (mg/L)	Temperature (° C)	pH (SU)	Dissolved Oxygen (mg/L)	Conductivity (µS/cm)
Control	0	23.9 ± 0.6 (23.5 - 24.4)	8.09 ± 0.24 (7.92 - 8.26)	7.5 ± 0.2 (7.4 - 7.7)	278 ± 4 (275 - 281)
6%	6.3	24.0 ± 0.7 (23.6 - 24.5)	8.05 ± 0.20 (7.91 - 8.19)	7.6 ± 0.5 (7.3 – 8.0)	280 ± 4 (277 - 282)
13%	12.5	23.9 ± 0.7 (23.4 - 24.4)	8.07 ± 0.18 (7.94 - 8.19)	7.7 ± 0.4 (7.5 – 8.0)	284 ± 10 (277 - 291)
25%	25.0	24.0 ± 0.6 (23.5 - 24.39)	8.08 ± 0.13 (7.98 - 8.17)	7.8 ± 0.3 (7.6 – 8.0)	285 ± 8 (279 - 290)
50%	50.0	24.0 ± 0.6 (23.6 - 24.4)	8.03 ± 0.14 (7.93 - 8.13)	7.7 ± 0.5 (7.3 – 8.0)	288 ± 8 (282 - 293)
100%	100.0	24.1 ± 0.4 (23.8 - 24.3)	8.03 ± 0.10 (7.96 - 8.1)	7.7 ± 0.3 (7.5 - 7.9)	303 ± 21 (288 - 318)

Table A4. Water quality parameters for the definitive, acute (48-hr) *Ceriodaphnia dubia* bioassay. Means and one standard deviation from the mean are indicated, with the minimum and maximum range of the data provided in parentheses.

Nominal Treatment (%)	Nominal Concentration (mg/L)	Temperature (° C)	pH (SU)	Dissolved Oxygen (mg/L)	Conductivity (µS/cm)
Control	0	23.1 ± 0.6 (22.7 - 23.5)	8.17 ± 0.13 (8.08 - 8.26)	8.2 ± 0.7 (7.7 - 8.6)	280 ± 7 (275 - 285)
6%	6.3	23.2 ± 0.5 (22.9 - 23.6)	8.18 ± 0.01 (8.17 - 8.19)	8.2 ± 0.4 (8.0 - 8.5)	283 ± 8 (277 - 288)
13%	12.5	23.2 ± 0.3 (23.0 - 23.4)	8.20 ± 0.01 (8.19 - 8.20)	8.2 ± 0.3 (8.0 - 8.4)	282 ± 7 (277 - 287)
25%	25.0	23.2 ± 0.4 (22.9 - 23.5)	8.18 ± 0.01 (8.17 - 8.19)	8.2 ± 0.2 (8.0 - 8.3)	285 ± 8 (279 - 290)
50%	50.0	23.3 ± 0.5 (22.9 - 23.6)	8.15 ± 0.03 (8.13 - 8.17)	8.1 ± 0.2 (8.0 - 8.3)	288 ± 8 (282 - 294)
100%	100.0	23.2 ± 0.8 (22.7 - 23.8)	8.14 ± 0.06 (8.10 - 8.18)	8.1 ± 0.2 (7.9 - 8.3)	294 ± 8 (288 - 299)

Table A5. Water quality parameters for the chronic (7-day) *Pimephales promelas* bioassay for in-water (a) and out-water (b) samples. Means and one standard deviation from the mean are indicated, with the minimum and maximum range of the data provided in parentheses.

Nominal Treatment (%)	Nominal Concentration (mg/L)	Temperature (° C)	pH (SU)	Dissolved Oxygen (mg/L)	Conductivity (µS/cm)
(a)					
Control	0	23.9 ± 0.4 (23.5 - 24.5)	7.98 ± 0.17 (7.70 - 8.18)	7.3 ± 0.5 (6.6 – 8.0)	274 ± 20 (252 - 311)
6%	1.6	23.9 ± 0.3 (23.6 - 24.5)	7.99 ± 0.12 (7.82 - 8.15)	7.4 ± 0.5 (6.6 – 8.0)	275 ± 17 (257 - 302)
13%	3.1	23.9 ± 0.3 (23.6 - 24.4)	7.99 ± 0.11 (7.84 - 8.17)	7.6 ± 0.3 (7.0 - 7.9)	273 ± 18 (252 - 302)
25%	6.3	23.9 ± 0.3 (23.6 - 24.4)	7.99 ± 0.1 (7.87 - 8.16)	7.6 ± 0.3 (7.1 - 7.9)	273 ± 18 (253 - 301)
50%	12.5	23.9 ± 0.3 (23.6 - 24.4)	7.98 ± 0.09 (7.88 - 8.14)	7.6 ± 0.2 (7.2 - 7.9)	273 ± 16 (254 - 302)
100%	25.0	23.9 ± 0.3 (23.5 - 24.4)	8.01 ± 0.09 (7.88 - 8.14)	7.7 ± 0.2 (7.3 – 8.0)	272 ± 17 (255 - 304)
(b)					
Control	0	24.0 ± 0.3 (23.6 - 24.6)	7.72 ± 0.10 (7.58 - 7.88)	6.0 ± 0.6 (5.1 - 6.9)	287 ± 25 (261 - 329)
6%	1.6	24.0 ± 0.3 (23.6 - 24.6)	7.65 ± 0.11 (7.52 - 7.83)	6.2 ± 0.5 (5.4 - 6.9)	287 ± 25 (264 - 330)
13%	3.1	24.0 ± 0.3 (23.5 - 24.6)	7.66 ± 0.13 (7.50 - 7.80)	6.2 ± 0.5 (5.4 - 6.8)	287 ± 24 (263 - 329)
25%	6.3	24.0 ± 0.4 (23.4 - 24.5)	7.64 ± 0.13 (7.49 - 7.79)	6.1 ± 0.5 (5.5 - 6.7)	286 ± 25 (262 - 329)
50%	12.5	24.0 ± 0.4 (23.3 - 24.5)	7.62 ± 0.12 (7.47 - 7.79)	6.3 ± 0.5 (5.4 - 6.8)	287 ± 24 (263 - 328)
100%	25.0	23.9 ± 0.4 (23.2 - 24.5)	7.62 ± 0.13 (7.48 - 7.78)	6.1 ± 0.5 (5.6 - 6.8)	288 ± 25 (267 - 328)

Table A6. Water quality parameters for the chronic (7-day) *Ceriodaphnia dubia* bioassay for in-water (a) and out-water (b) samples. Means and one standard deviation from the mean are indicated, with the minimum and maximum range of the data provided in parentheses.

Nominal Treatment (%)	Nominal Concentration (mg/L)	Temperature (° C)	pH (SU)	Dissolved Oxygen (mg/L)	Conductivity (µS/cm)
(a)					
Control	0	23.9 ± 0.4 (23.5 - 24.5)	7.98 ± 0.17 (7.70 - 8.18)	7.3 ± 0.5 (6.6 – 8.0)	274 ± 20 (252 - 311)
6%	1.6	23.9 ± 0.3 (23.6 - 24.5)	7.99 ± 0.12 (7.82 - 8.15)	7.4 ± 0.5 (6.6 – 8.0)	275 ± 17 (257 - 302)
13%	3.1	23.9 ± 0.3 (23.6 - 24.4)	7.99 ± 0.11 (7.84 - 8.17)	7.6 ± 0.3 (7.0 - 7.9)	273 ± 18 (252 - 302)
25%	6.3	23.9 ± 0.3 (23.6 - 24.4)	7.99 ± 0.1 (7.87 - 8.16)	7.6 ± 0.3 (7.1 - 7.9)	273 ± 18 (253 - 301)
50%	12.5	23.9 ± 0.3 (23.6 - 24.4)	7.98 ± 0.09 (7.88 - 8.14)	7.6 ± 0.2 (7.2 - 7.9)	273 ± 16 (254 - 302)
100%	25.0	23.9 ± 0.3 (23.5 - 24.4)	8.01 ± 0.09 (7.88 - 8.14)	7.7 ± 0.2 (7.3 – 8.0)	272 ± 17 (255 - 304)
(b)					
Control	0	24.1 ± 0.2 (23.9 - 24.3)	8.01 ± 0.09 (7.94 - 8.14)	7.3 ± 0.7 (6.3 - 7.9)	284 ± 21 (262 - 312)
6%	1.6	24.3 ± 0.2 (24.0 - 24.6)	7.99 ± 0.05 (7.90 - 8.03)	7.3 ± 0.5 (6.6 - 7.8)	289 ± 25 (257 - 317)
13%	3.1	24.2 ± 0.3 (23.7 - 24.6)	7.97 ± 0.06 (7.90 - 8.05)	7.3 ± 0.5 (6.5 - 7.9)	288 ± 24 (257 - 316)
25%	6.3	24.2 ± 0.3 (23.8 - 24.6)	7.95 ± 0.07 (7.84 - 8.02)	7.4 ± 0.6 (6.5 – 8.0)	290 ± 24 (260 - 317)
50%	12.5	24.2 ± 0.3 (23.8 - 24.6)	7.92 ± 0.07 (7.81 - 7.99)	7.3 ± 0.6 (6.3 - 7.9)	288 ± 26 (255 - 317)
100%	25.0	24.1 ± 0.2 (23.8 - 24.4)	7.90 ± 0.07 (7.78 - 7.98)	7.4 ± 0.6 (6.5 - 7.9)	289 ± 26 (257 - 315)

Appendix B: Statistical Analysis

Acute Fish Test-48 Hr Survival

Start Date:	2/1/2012	Test ID:	1	Sample ID:	DNAN	
End Date:	2/3/2012	Lab ID:		Sample Type:		
Sample Dat		Protocol:	EPAA 91-EPA Acute	Test Species:	PP-Pimephales promelas	
Comments:						

Conc-ug/L	1	2	3	4
0.1	1.0000	1.0000	1.0000	1.0000
7.1	1.0000	1.0000	1.0000	1.0000
13	1.0000	1.0000	1.0000	1.0000
28	0.8000	0.7000	0.8000	0.7000
74	0.0000	0.0000	0.0000	0.0000
120	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed		
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical		
0.1	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4				
7.1	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00		
13	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00		
*28	0.7500	0.7500	1.0492	0.9912	1.1071	6.383	4	10.00	10.00		
*74	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	4	10.00	10.00		
*120	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	4	10.00	10.00		

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.5762328	0.884	6.572E-15	4.0324675
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	13	28	19.078764	

Acute Fish Test-48 Hr Survival

Start Date:	2/1/2012	Test ID:	1	Sample ID:	DNAN	
End Date:	2/3/2012	Lab ID:		Sample Type:		
Sample Dat		Protocol:	EPAA 91-EPA Acute	Test Species:	PP-Pimephales promelas	
Comments:						

Conc-ug/L	1	2	3	4
0.1	1.0000	1.0000	1.0000	1.0000
7.1	1.0000	1.0000	1.0000	1.0000
13	1.0000	1.0000	1.0000	1.0000
28	0.8000	0.7000	0.8000	0.7000
74	0.0000	0.0000	0.0000	0.0000
120	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Number	Total
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Resp	Number
0.1	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	0	40
7.1	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	0	40
13	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	0	40
28	0.7500	0.7500	1.0492	0.9912	1.1071	6.383	4	10	40
74	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	4	40	40
120	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	4	40	40

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.5762328	0.884	6.572E-15	4.0324675
Equality of variance cannot be confirmed				

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%	36.626	32.514	41.257
5.0%	37.217	32.539	42.566
10.0%	37.759	32.245	44.216
20.0%	38.570	30.140	49.356
Auto-0.0%	36.626	32.514	41.257

Acute Fish Test-48 Hr Survival

Start Date:	2/1/2012	Test ID:	1	Sample ID:	DNAN	
End Date:	2/3/2012	Lab ID:		Sample Type:		
Sample Dati		Protocol:	EPAA 91-EPA Acute	Test Species:	CD-Ceriodaphnia dubia	
Comments:						

Conc-ug/L	1	2	3	4
0.1	0.6000	1.0000	1.0000	1.0000
7.1	0.8000	1.0000	1.0000	1.0000
13	0.8000	0.8000	0.8000	0.8000
28	1.0000	0.8000	1.0000	1.0000
74	0.0000	0.0000	0.0000	0.0000
120	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed			
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical			
0.1	0.9000	1.0000	1.2305	0.8861	1.3453	18.660	4					
7.1	0.9500	1.0556	1.2857	1.1071	1.3453	9.261	4	18.50	10.00			
13	0.8000	0.8889	1.1071	1.1071	1.1071	0.000	4	14.00	10.00			
28	0.9500	1.0556	1.2857	1.1071	1.3453	9.261	4	18.50	10.00			
*74	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4	10.00	10.00			
*120	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4	10.00	10.00			

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.7342137	0.884	-2.025649	4.998213
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	28	74	45.519227	

Acute Fish Test-48 Hr Survival

Start Date:	2/1/2012	Test ID:	1	Sample ID:	DNAN	
End Date:	2/3/2012	Lab ID:		Sample Type:		
Sample Dati		Protocol:	EPAA 91-EPA Acute	Test Species:	CD-Ceriodaphnia dubia	
Comments:						

Conc-ug/L	1	2	3	4
0.1	0.6000	1.0000	1.0000	1.0000
7.1	0.8000	1.0000	1.0000	1.0000
13	0.8000	0.8000	0.8000	0.8000
28	1.0000	0.8000	1.0000	1.0000
74	0.0000	0.0000	0.0000	0.0000
120	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Number	Total
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Resp	Number
0.1	0.9000	1.0000	1.2305	0.8861	1.3453	18.660	4	2	20
7.1	0.9500	1.0556	1.2857	1.1071	1.3453	9.261	4	1	20
13	0.8000	0.8889	1.1071	1.1071	1.1071	0.000	4	4	20
28	0.9500	1.0556	1.2857	1.1071	1.3453	9.261	4	1	20
74	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4	20	20
120	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4	20	20

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.7342137	0.884	-2.025649	4.998213
Equality of variance cannot be confirmed				

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%	41.848	37.414	46.808
5.0%	44.116	39.571	49.183
10.0%	44.273	41.907	46.772
20.0%	44.273	41.907	46.772
Auto-0.0%	41.848	37.414	46.808

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	3/23/2012	Test ID:	1	Sample ID:	DNAN
End Date:	3/30/2012	Lab ID:	ERDC	Sample Type:	
Sample Dat		Protocol:	EPAF 94-EPA Freshwater	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2	3	4	5
0.1934786	1.0000	1.0000	0.9000	1.0000	1.0000
0.67	1.0000	1.0000	1.0000	1.0000	1.0000
1.375	0.9000	1.0000	1.0000	1.0000	1.0000
2.5469231	1.0000	1.0000	0.9000	1.0000	1.0000
5.7642857	0.7000	1.0000	0.9000	0.9000	1.0000
11.607143	0.4000	0.3000	0.4000	0.4000	0.4000
24.555556	0.0000	0.0000	0.0000	0.0000	0.0000

Conc-mg/L	Mean	N-Mean	Resp	Not Resp	Total	N	Fisher's Exact P	1-Tailed Critical
0.1934786	1.0000	1.0000	0	5	5	5		
0.67	1.0000	1.0000	0	5	5	5	1.0000	0.0500
1.375	1.0000	1.0000	0	5	5	5	1.0000	0.0500
2.5469231	1.0000	1.0000	0	5	5	5	1.0000	0.0500
5.7642857	1.0000	1.0000	0	5	5	5	1.0000	0.0500
1428571429	0.0000	0.0000	5	0	5	5	0.0040	0.0500
5555555556	0.0000	0.0000	5	0	5	5	0.0040	0.0500

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Fisher's Exact Test	5.7642857	11.607143	8.1796631	

Larval Fish Growth and Survival Test-7 Day Biomass

Start Date:	3/23/2012	Test ID:	1	Sample ID:	DNAN
End Date:	3/30/2012	Lab ID:		Sample Type:	
Sample Dat		Protocol:	EPAF 94-EPA Freshwater	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2	3	4	5
0.1934786	0.4022	0.3958	0.4949	0.3638	0.4302
0.67	0.4294	0.3702	0.4186	0.4626	0.4078
1.375	0.3882	0.4258	0.4692	0.5462	0.4402
2.5469231	0.3952	0.4114	0.4920	0.5320	0.3518
5.7642857	0.5391	0.4894	0.4460	0.3902	0.3944
11.607143	0.2115	0.4207	0.3095	0.2695	0.2875
24.555556	0.0000	0.0000	0.0000	0.0000	0.0000

			Transform: Untransformed					Rank	1-Tailed
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical
0.1934786	0.4214	1.0000	0.4214	0.3638	0.4949	10.557	5		
0.67	0.4177	0.9913	0.4177	0.3702	0.4626	8.037	5	28.00	16.00
1.375	0.4539	1.0772	0.4539	0.3882	0.5462	13.054	5	32.00	16.00
2.5469231	0.4365	1.0358	0.4365	0.3518	0.5320	16.879	5	28.00	16.00
5.7642857	0.4518	1.0723	0.4518	0.3902	0.5391	14.069	5	30.00	16.00
11.607143	0.2997	0.7113	0.2997	0.2115	0.4207	25.610	5	18.00	16.00
5555555556	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	5	15.00	16.00

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9494278	0.91	0.5891798	0.0808855
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	11.607143	24.555556	16.882531	

Larval Fish Growth and Survival Test-7 Day Biomass

Start Date:	3/23/2012	Test ID:	1	Sample ID:	DNAN
End Date:	3/30/2012	Lab ID:		Sample Type:	
Sample Dati		Protocol:	EPAF 94-EPA Freshwater	Test Species:	PP-Pimephales promelas
Comments:					

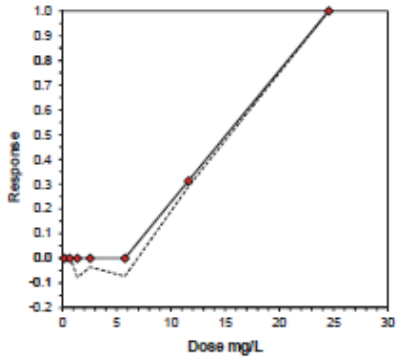
Conc-mg/L	1	2	3	4	5
0.1934786	0.4022	0.3958	0.4949	0.3838	0.4302
0.67	0.4294	0.3702	0.4186	0.4626	0.4078
1.375	0.3882	0.4258	0.4692	0.5462	0.4402
2.5469231	0.3962	0.4114	0.4920	0.5320	0.3518
5.7642857	0.5391	0.4894	0.4460	0.3902	0.3944
11.607143	0.2115	0.4207	0.3095	0.2695	0.2875
24.555556	0.0000	0.0000	0.0000	0.0000	0.0000

			Transform: Untransformed					Isotonic	
Conc-mg/l	Mean	N-Mean	Mean	Min	Max	CV%	N	Mean	N-Mean
0.1934786	0.4214	1.0000	0.4214	0.3838	0.4949	10.557	5	0.4363	1.0000
0.67	0.4177	0.9913	0.4177	0.3702	0.4626	8.037	5	0.4363	1.0000
1.375	0.4539	1.0772	0.4539	0.3882	0.5462	13.054	5	0.4363	1.0000
2.5469231	0.4365	1.0358	0.4365	0.3518	0.5320	16.879	5	0.4363	1.0000
5.7642857	0.4518	1.0723	0.4518	0.3902	0.5391	14.069	5	0.4363	1.0000
11.607143	0.2997	0.7113	0.2997	0.2115	0.4207	25.610	5	0.2997	0.6870
24.555556	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	5	0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9494278	0.91	0.5891798	0.0808855
Equality of variance cannot be confirmed				

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	6.698	1.381	0.000	8.015	-1.6947
IC10	7.631	0.941	5.293	10.339	2.4481
IC15	8.565	1.031	6.394	12.545	1.7874
IC20	9.498	1.130	7.420	13.584	1.3112
IC25	10.432	1.158	8.243	14.274	0.9380
IC40	13.248	1.143	10.093	16.334	0.2062
IC50	15.132	0.998	12.263	17.705	-0.0848



Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	3/23/2012	Test ID:	1	Sample ID:	DNAN	
End Date:	3/30/2012	Lab ID:	ERDC	Sample Type:		
Sample Date		Protocol:	EPAF 94-EPA Freshwater	Test Species:	CD-Ceriodaphnia dubia	
Comments:						

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
0.00124	39.000	40.000	30.000	0.000	41.000	39.000	32.000	43.000	44.000	40.000
0.7316667	32.000	37.000	33.000	42.000	37.000	36.000	40.000	48.000	39.000	40.000
1.4866667	39.000	43.000	32.000	38.000	42.000	43.000	32.000	38.000	38.000	38.000
3.12	43.000	36.000	34.000	31.000	34.000	45.000	40.000	46.000	43.000	45.000
6.2	35.000	43.000	38.000	35.000	38.000	35.000	34.000	34.000	33.000	37.000
12.166667	16.000	4.000	6.000	15.000	16.000	10.000	18.000	14.000	14.000	15.000
24.2	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000

			Transform: Untransformed					Rank	1-Tailed
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical
0.00124	34.800	1.0000	34.800	0.000	44.000	37.364	10		
0.7316667	38.400	1.1034	38.400	32.000	48.000	11.978	10	103.50	74.00
1.4866667	38.300	1.1006	38.300	32.000	43.000	10.228	10	101.00	74.00
3.12	39.700	1.1408	39.700	31.000	46.000	13.850	10	119.00	74.00
6.2	36.200	1.0402	36.200	33.000	43.000	8.111	10	90.50	74.00
5666666667	12.800	0.3678	12.800	4.000	18.000	36.047	10	65.00	74.00
*24.2	0.100	0.0029	0.100	0.000	1.000	316.228	10	60.50	74.00

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.01)	1.2781303	1.035	-2.996103	16.868357
Bartlett's Test indicates unequal variances (p = 2.51E-13)	71.030075	16.811893		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	6.2	12.166667	8.6852365	

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	3/23/2012	Test ID:	1	Sample ID:	DNAN	
End Date:	3/30/2012	Lab ID:	ERDC	Sample Type:		
Sample Date		Protocol:	EPAF 94-EPA Freshwater	Test Species:	CD-Ceriodaphnia dubia	
Comments:						

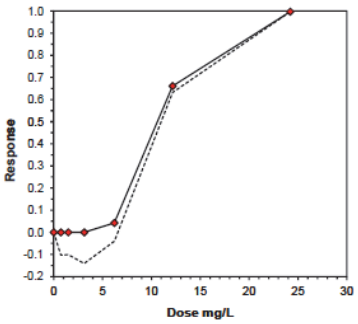
Conc-mg/L	1	2	3	4	5	6	7	8	9	10
0.00124	39.000	40.000	30.000	0.000	41.000	39.000	32.000	43.000	44.000	40.000
0.7316667	32.000	37.000	33.000	42.000	37.000	36.000	40.000	48.000	39.000	40.000
1.4866667	39.000	43.000	32.000	38.000	42.000	43.000	32.000	38.000	38.000	38.000
3.12	43.000	36.000	34.000	31.000	34.000	45.000	40.000	46.000	43.000	45.000
6.2	35.000	43.000	38.000	35.000	38.000	35.000	34.000	34.000	33.000	37.000
12.166667	16.000	4.000	6.000	15.000	16.000	10.000	18.000	14.000	14.000	15.000
24.2	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000

			Transform: Untransformed					Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Mean	N-Mean
0.00124	34.800	1.0000	34.800	0.000	44.000	37.364	10	37.800	1.0000
0.7316667	38.400	1.1034	38.400	32.000	48.000	11.978	10	37.800	1.0000
1.4866667	38.300	1.1006	38.300	32.000	43.000	10.228	10	37.800	1.0000
3.12	39.700	1.1408	39.700	31.000	46.000	13.850	10	37.800	1.0000
6.2	36.200	1.0402	36.200	33.000	43.000	8.111	10	36.200	0.9577
12.166667	12.800	0.3678	12.800	4.000	18.000	36.047	10	12.800	0.3386
24.2	0.100	0.0029	0.100	0.000	1.000	316.228	10	0.100	0.0026

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.01)	1.2781303	1.035	-2.996103	16.868357
Bartlett's Test indicates unequal variances (p = 2.51E-13)	71.030075	16.811893		

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	6.274	1.519	0.701	6.687	-1.8852
IC10	6.756	0.572	5.027	7.174	-1.9862
IC15	7.238	0.348	6.391	7.670	-0.6433
IC20	7.720	0.328	6.952	8.160	-0.5450
IC25	8.202	0.313	7.440	8.650	-0.4736
IC40	9.647	0.292	9.023	10.141	-0.1795
IC50	10.611	0.300	9.998	11.193	-0.0049



Appendix C: Laboratory Bioassay Bench Sheets

Laboratory bioassay bench test sheets for the work documented in this report can be obtained by contacting:

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U.S. Army Engineer Research and Development Center
3909 Halls Ferry Road
Vicksburg, MS 39180-6199

601-634-3344

Alan.J.Kennedy@usace.army.mil

Appendix D. Analytical reports

Analytical reports for the work documented in this report can be obtained by contacting:

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14. ABSTRACT

Insensitive munition formulation (IMX)-101 consists of 2,4-dinitroanisole (DNAN), 3-nitro-1,2,4-triazol-5-one (NTO), and nitroguanidine (NQ). While general aquatic ecotoxicological information is available for two of the IMX constituents (NTO and NQ), such data are not known to be available for DNAN. Thus, acute and chronic aquatic toxicity bioassays were conducted using standard fish (*Pimephales promelas*) and invertebrate (*Ceriodaphnia dubia*) models. Chemical analysis of test water indicated that DNAN concentrations were relatively stable during the bioassays. Acute toxicity was similar for the two species tested, with 48-hr lethal median concentrations (LC50) ranging from 37 to 42 mg/L DNAN. Chronic toxicity tests indicated that fish survival (7-day LC50 = 10 mg/L) was significantly more sensitive to DNAN relative to the invertebrate (no significant impact on survival at 24 mg/L). However, the reproduction endpoint for the invertebrate was significantly more sensitive to DNAN than survival. When assessing the most sensitive chronic endpoints, the two test species indicated similar chronic toxicity, with lowest observable adverse impacts ranging from 10 to 12 mg/L DNAN and median effects on sublethal endpoints (growth, reproduction) ranging from 11 to 15 mg/L DNAN. Chronic no-effect concentrations ranged from approximately 6 to 8 mg/L DNAN.

15. SUBJECT TERMS

2,4 Dinitroanisole
Acute toxicity testing
Aquatic ecotoxicological exposures
Chronic toxicity testing
Insensitive munitions

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